

# The eating quality of beef from young dairy bulls derived from two breed types at three ages from two different production systems

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## Abstract

Expansion of the Irish dairy herd has led to more dairy breed male calves being available for beef production. This study investigated the physico-chemical and sensory characteristics of beef from Holstein–Friesian (HF) and Jersey × HF (JEX) young bulls fed pasture grass only or pasture grass plus 2 kg concentrate during their first grazing season and slaughtered at 15, 19 or 22 mo of age. *Longissimus thoracis* (LT) muscles were collected from 67 carcasses. Post-mortem pH, ultimate pH (pHu), meat colour, chemical composition, collagen content and solubility were evaluated. After ageing for 21 d, Warner-Bratzler shear force and cooking loss were determined, and assessments by a trained sensory panel were conducted. Meat from older animals was darker. The pHu, moisture and ash contents decreased, while residual roast beef flavour length increased with age. However, increasing age to slaughter did not negatively influence tenderness. JEX beef had lower cooking loss, was darker and redder, in addition to having higher sensory scores for initial tenderness and fattiness than HF beef. Warner-Bratzler variables were positively correlated with cooking loss and chewiness and were negatively correlated with intramuscular fat (IMF) content, soluble collagen and initial tenderness. In summary, most young dairy bull beef samples were acceptably tender after 21 d of ageing and half of them had acceptable IMF content. Slaughter age affected beef colour, pHu, chemical composition and flavour length. The eating quality of meat from the JEX breed type was considered to be superior to that of the HF breed type. Diet during the first season had no effect on meat quality traits.

## Keywords

dairy breeds • meat quality • sensory attributes • tenderness • young bulls

## Introduction

Meat quality is a complex concept that involves intrinsic quality cues, including safety, shelf life, nutritional value and eating quality, as well as extrinsic quality cues, such as brand, quality label and convenience of the product (Hocquette *et al.*, 2012). Eating quality is generally considered the most important meat quality trait for consumer satisfaction, with juiciness, tenderness and flavour being the major attributes (Troy and Kerry, 2010). Marbling is another important intrinsic factor that contributes to beef palatability and hence is used as an indicator for beef quality grading (Li *et al.*, 2006).

The ending of milk quotas in 2015 has led to increased dairy output in Ireland. The Food Harvest 2020 Strategy predicted an increase in the dairy herd from 1.15 million to 1.43 million by 2020 (DAFM, 2014). The number of male calves from the dairy herd is therefore rising. Raising these calves as steers is not a viable option due to their poor conformation, while rearing them as young bulls may be a more viable option due to their

improved growth rate and feed conversion efficiency, as well as subsequent lower carbon emissions compared to steers and the probability of generating viable financial returns (Seideman *et al.*, 1982). Such an approach would provide a significant new source of income for producers, increase beef supplies and potentially open up new export markets for Irish beef. However, although young bull beef production has increased in the past decade, it still only accounts for 19% of the overall Irish male cattle slaughtered in 2015, down from 22% in 2014, 25% in 2013 and 30% in 2012 (DAFM, 2015). There is conflicting evidence about the palatability of bull beef, and there is a view in the industry that it is tougher than steer beef and, therefore, less acceptable to consumers. There is little recent information on the palatability of beef from young dairy bulls and how this is affected by the production system utilised. This study addresses this knowledge gap.

Meat attributes such as colour, water-holding capacity (WHC) and tenderness can be affected by production system factors,

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including breed, feeding regime, slaughter age, handling and exercise conditions (Frylinck *et al.*, 2013). Holstein–Friesian (HF) is the predominant Irish dairy breed; however, there is currently interest in the Jersey breed as these have shown potential for crossbreeding under Irish conditions, due to their improved reproductive efficiency, increased intake capacity and increased yields of milk solids (Prendiville *et al.*, 2011). Growth rate, carcass traits and performance of HF and JEX bulls were recently reported (McNamee *et al.*, 2015); however, palatability has not been well investigated and there are concerns about the eating quality of bull beef in general. Moreover, cattle age is known to closely correlate with the physico-chemical characteristics of meat and is an important factor in determining meat tenderness and palatability (Schönfeldt and Strydom, 2011). Beef quality from pasture- or grain-based finishing systems has been extensively researched (French *et al.*, 2000; Avilés *et al.*, 2015). It has been shown that the high-energy diet produced higher levels of intramuscular fat (IMF) and flavour acceptance of beef compared with the low-energy diet (Corbin *et al.*, 2015), while the effect of the first season (grazing phase) on beef quality has not been explored. It is hypothesised that beef produced from a higher-energy diet during the first grazing season could exhibit higher potential for marbling deposition than that from a lower-energy diet.

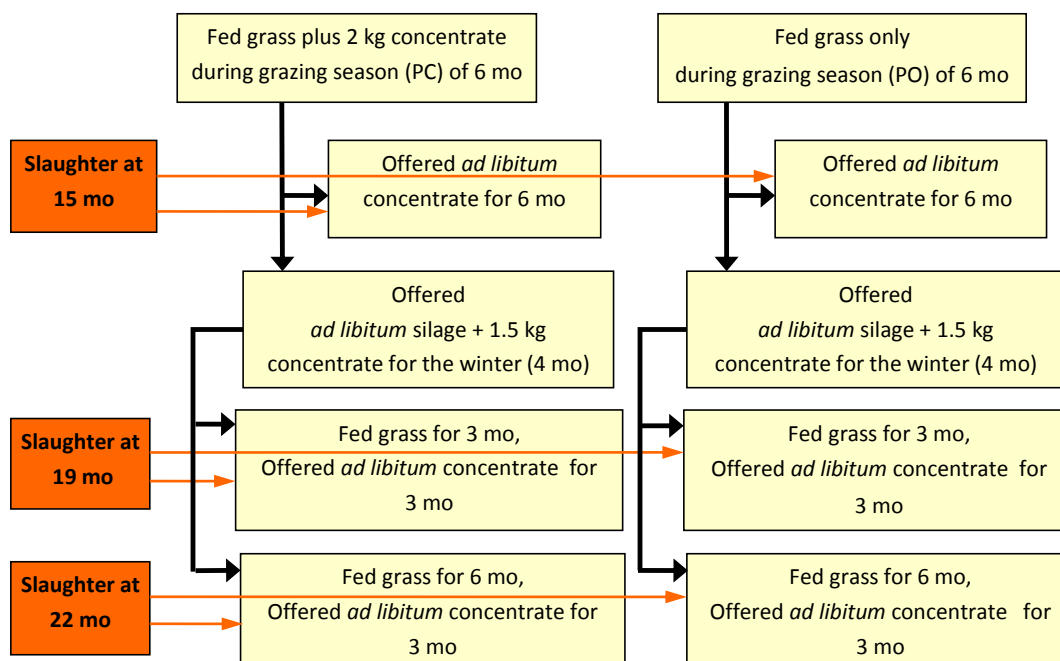
This study aims to determine the physico-chemical and sensory characteristics of beef from young bulls of two dairy breed types, slaughtered at three ages from two different

production systems. A greater understanding of how breed types, slaughter age and first-season feeding affect dairy bull production efficiency and beef quality attributes would assist in decision-making on how meat from dairy bulls should be produced and marketed.

## Materials and methods

### Source of materials

This project was submitted to the Teagasc Animal Ethics Committee, which advised that provided best husbandry practice was followed, no ethical issues would arise. A total of 300 (mainly HF & JEX) weaned, spring-born male dairy breed type calves (10–12 wk of age) were sourced and transported to Teagasc, Johnstown Castle Research Centre, in 2010. Then, they were assigned to one of two production systems (pasture grass only: PO vs. pasture grass plus 2 kg concentrate: PC) during the first grazing season according to breed type, date of birth, body weight on arrival and farm of origin. The concentrates offered per head daily was composed of 80% *Hordeum vulgare* (ground barley), 14% *Glycine max* (L.) Merr. (soybean meal), 4% black treacle (molasses) and 2% minerals. Bulls were slaughtered at 15, 19 and 22 mo of age (Figure 1). The experiment was set up as a 3 (slaughter age) × 2 (breed type) × 2 (first-season feeding) factorial design, resulting in 12 treatment groups.



**Figure 1.** Production treatments of young dairy bulls. HF = Holstein–Friesian; JEX = Jersey × HF.

Permanent grassland sward of predominantly perennial ryegrass (*Lolium perenne*) was used for rotational grazing systems. Animals assigned to PO or PC (excluding 15-mo-old bulls) were housed together during the winter period within their respective production systems. Before finishing, 19- and 22-mo-old bulls were also offered the second grazing season with a grass-only diet. During the finishing period, animals were penned within their own treatment group and offered an *ad libitum* concentrate diet. The duration of each feeding treatment is shown in Figure 1.

### Sampling and sample preparation

At a commercial abattoir, bulls were stunned by a captive bolt stunner and exsanguinated within 30 s. Electrical stimulation was not applied. Each carcass was conventionally hung, dressed and centrally split into two sides. The pH and temperature of the *longissimus thoracis* (LT) muscle at the 10th rib on the left side of each carcass were measured hourly for up to 8 h. Carcasses were chilled at 4°C. A sub-sample of each batch was selected for meat quality analysis. As there were many more HF than JEX bulls, all suitable (normal pH and not detained for veterinary inspection) JEX and up to 10 HF carcasses per treatment group were selected. In total, 67 bulls were sampled, 33 from PO and 34 from PC; of these, 39 were from HF and 28 were from JEX; furthermore, 29 were slaughtered at 15 mo, 19 at 19 mo and 19 at 22 mo.

The LT muscle was removed from the cube roll (ribs 6–10) from the left side of each selected carcass at 48 h post mortem. After holding until 72 h post mortem, the ultimate pH (pHu) of the LT samples was measured, and the muscle was cut into individual slices (25 mm thick). The fresh-cut surface of the first slice from the 10th rib end was used for colour measurement, and the remaining slices were vacuum-packed. Steaks for determination of chemical composition and collagen were stored immediately at –20°C, while samples for determination of Warner-Bratzler shear force (WBSF) and cooking loss, as well as sensory analysis, were aged for 21 d at 4°C and then frozen at –20°C for further analysis.

### Post-mortem pH, temperature and pHu

A portable pH meter (model 420A; Orion, Hamburg, Germany) and an Amagross pH electrode EC-2010-11 (Refex Sensors Ltd., Westport, Co. Mayo, Ireland) were calibrated using standard buffer solutions (pH 4.0 and pH 7.0). The temperature probe (Digitron 2046T; Instrument Technology Ltd, Dunboyne, Co. Meath, Ireland) and pH electrode were inserted approximately 50 mm into the LT muscle near the 10th rib following a scalpel incision. The electrode was rinsed thoroughly with distilled water between measurements. The pH/temperature profiles of each carcass were prepared using the pH and temperature values measured up to 8 h post mortem. The individual pH values at 15°C and 35°C of

each carcass were then read from the pH/temperature curves directly.

### Meat colour

Freshly cut samples were wrapped with an oxygen-permeable polyvinylchloride film (oxygen permeability of 580 mL/m<sup>2</sup> per hour) and left to bloom at 4°C for 2 h and 24 h. Measurements were taken through the film at five locations on each sample and averaged using a dual-beam spectrophotometer (UltraScan XE, Hunter Lab., Reston, VA, USA) with a wavelength range from 360 to 750 nm and a wavelength interval of 5 nm. A light trap and a white tile were used for standardisation. Illumination was matched to daylight (D65, 10°) with an 8° viewing angle and a 25 mm port size. The Commission Internationale d'Eclairage or International Commission on Light (CIE) L\*(lightness), a\*(redness) and b\*(yellowness) values were recorded. The hue angle ( $\tan^{-1}(b^*/a^*) \times 57.29$ ) and the saturation index ( $(a^{*2} + b^{*2})^{1/2}$ ) were calculated.

### WBSF and cooking loss

Trimmed steaks were thawed in a circulating water bath at 10–15°C. Excess moisture was removed by patting the surfaces of steaks with tissue paper before weighing. The steaks were cooked in open bags suspended in a water bath at 72°C until the temperature in the centre of the steak reached 70°C. When the steaks had cooled to room temperature, they were again patted with tissue paper and weighed. Cooking loss was determined as the difference between the cooked and raw weights expressed as a percentage of the raw weight.

After tempering overnight at 4°C, seven cores of 12.5 mm diameter per steak were cut parallel to the longitudinal orientation of the muscle fibres. When the cores reached room temperature (approximately 20°C), they were sheared perpendicular to the muscle fibre orientation using the Warner-Bratzler (WB) shear blade attached to an Instron universal testing machine [Model 5543, Instron (UK) Ltd., High Wycombe, Buckinghamshire, UK] with a 500 N load cell using a cross-head speed of 50 mm/min. The average maximum shear force (WBSF) was calculated by excluding the two extreme values. WB slope was recorded from a line drawn from 20% to 80% of the WBSF curve and expressed as megapascals (Mpa), and the WB area was calculated by the whole energy used during shearing and expressed as joules (J).

### Chemical composition

Frozen samples, trimmed of all external fat and connective tissue, were thawed at 4°C overnight. The lean meat and exudate were homogenised using a blender (R301 Ultra; Robot Coupe SA, Vincennes, France). The moisture and IMF concentrations of the thawed minced beef samples were measured using the Smart System 5 microwave drying oven and nuclear magnetic resonance (NMR) Smart Trac rapid fat

analyser (CEM Corporation, Matthews, NC, USA) using the official method 985.14 of the Association of Official Analytical Chemists (AOAC 985.14, 1991). Protein concentration was determined using a LECO FP328 protein analyser (LECO Corp., St. Joseph, MI, USA) based on the Dumas method and according to AOAC method 992.15 (AOAC 992.15, 1992). Approximately 2–3 g of homogenised sample was weighed in a crucible dish, which was placed on the electric hot plate until charred. The crucible dish was then transferred to a muffle furnace (515–540°C) and left overnight until formation of ash. Samples were removed from the furnace, cooled to room temperature and reweighed to determine the ash percentage. All tests for composition were carried out as two determinations per sample, with the coefficient of variation (CV) between replicates of moisture content <1.0%; of IMF content <10%; of protein content <1.5%.

### **Collagen content and solubility**

According to a combination of the methods of Voutilainen *et al.* (2007), Kolar (1990) and the Nordic Committee on Food Analysis (2002), 5 g of homogenised meat was heated in a water bath at 77°C for 65 min in 12 mL of buffer solution (pH 6.0; 30 g citric acid monohydrate, 15 g NaOH and 90 g sodium acetate trihydrate dissolved in 290 mL 1-propanol and diluted to 1 L with water) and centrifuged for 10 min at  $3,990 \times g$  (MSE Mistral 3000i; MSE UK Ltd., London, UK) at room temperature. The supernatant was collected and 8 mL of buffer solution was mixed with the precipitate and centrifuged again for another 10 min. The precipitates and supernatants from the two centrifugations were respectively combined. Each fraction was individually hydrolysed in 30 mL of 7 N  $H_2SO_4$  in an oven at 105°C for 17 h. The hot hydrolysate was transferred to a 250 mL volumetric flask with the aid of water, and the solution was neutralised with 4.37 mL of 1 M NaOH, diluted to volume with water and mixed well. Part of the solution was passed through filter paper into a 100 mL Erlenmeyer flask. A total of 5 mL of the filtrate was diluted to 100 mL. To 2 mL of the final dilution, 1 mL of oxidant solution (1.41 g chloramine-T reagent in 100 mL buffer solution) was added and left to stand for approximately 20 min at room temperature. Then, 1 mL of colouring reagent (10 g of 4-dimethylaminobenzaldehyde dissolved in 35 mL perchloric acid [60%, w/w] and 65 mL 2-propanol) was added and the tubes were immediately placed in a water bath at  $60 \pm 0.5^\circ C$  for 15 min. The tubes were cooled and dried, and the absorbance was measured at  $558 \pm 2$  nm with an ultraviolet spectrophotometer UV-1700 (Shimadzu, Columbia, MD, USA). Soluble and insoluble collagen contents were calculated by multiplying the hydroxyproline amount by a factor of 7.25. Total collagen (milligrams collagen per gram meat) was defined as the sum of soluble collagen and insoluble collagen, and percentage solubility was calculated

as soluble collagen as a percentage of total collagen. Each sample was analysed in duplicate, and the CV value between duplicates was <10%.

### **Evaluation by the trained sensory panel**

Frozen vacuum-packed steaks of 25 mm thickness were thawed in a circulating water bath at 10°C. Meat was heated to an internal temperature of 70°C on the lower plate of a double-contact electric grill (Velox CG-3, Velox Grills, Wantage, Oxfordshire, UK) set at 230°C, according to the guidelines of the American Meat Science Association (AMSA, 1978). Temperature was monitored with a probe (Eurolec TH103TC; Technology House, Dundalk, Co. Louth, Ireland) inserted into the centre of each steak. Steaks were grilled for 1 min on one side, turned over for 1 min and turned twice more for 2 min, followed by continuous turning each minute until done. Steaks were then trimmed of all external fat and major connective tissue, cut into pieces (20 × 15 × 25 mm) and wrapped in aluminium foil for resting for about 3 min and served to an eight-member trained sensory panel seated in individual booths with red fluorescent light. Each panel member, trained according to AMSA (1995) standards, received six samples in randomised order (each panellist tasted the steak samples in a different order within each session) in two sets of three, with approximately 3 min intervals between each set. Panel members were provided with salt-free crackers and water for cleansing the palate between samples.

Panellists scored each sample for 16 attributes, defined and rated during different phases of eating (Table 1). Roast beef aroma intensity was evaluated before eating, while the initial tenderness was the texture of the first bite. During further mastication, juiciness, cohesiveness, ease of disintegration, chewiness, fattiness/greasiness, stringiness, astringency and the flavour terms “roast beef flavour”, “metallic” and “stale/rancid/aged” were evaluated. Residual roast beef flavour length, residual metallic flavour, residual fattiness/greasiness and residual dryness were the sensations left in the mouth 12 s after swallowing the sample, thus they were described as residual or aftereffects. Each attribute was rated through “Compusense® five” sensory evaluation software (Compusense Inc., Guelph, ON, Canada) on station computers, by a 100 mm unstructured line scale with 0 mm being equivalent to no intensity and 100 mm being equivalent to the highest intensity of the attribute.

### **Statistical analysis**

The data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System (SAS, 2002). The experimental unit was the individual animal for all variables, to keep the unit consistent throughout the whole feeding period. The model included the fixed effects of breed type (HF and JEX), age at slaughter

**Table 1.** Definition of sensory attributes of *longissimus thoracis* muscles of young dairy bulls

Attributes	Definitions	Scale anchors <sup>1</sup>
<b>Aroma</b>		
Roast beef aroma	Intensity of roast beef aroma	Weak–strong
Cut beef sample into bite-sized pieces. Put a bite-sized piece into the mouth and assess the resistance to the teeth (molars)		
<b>Texture</b>		
Initial tenderness	The resistance to teeth biting through the sample	Not–very
<b>During eating</b>		
Juiciness	In the first 3–4 chews, the moisture released from the sample	Not–very
Cohesiveness	In the first 3–4 chews, how well the sample holds its structure	Not–very
Ease of disintegration	After 6–7 chews, the ease with which the meat breaks down into particles in the mouth	Not easily–easily
Chewiness	Number of chews/the force required to break down the sample to swallow	Not–very
Fattiness/greasiness	Fatty film on the inside of the mouth during mastication	Not–very
<b>Just before swallowing</b>		
Stringiness	Sensation of strings during mastication	Not–very
Astringency	Drying effect in the mouth during mastication	Not–very
Using a new bite-sized piece of beef, assess the flavour and aftereffect attributes.		
<b>Flavour</b>		
Roast beef flavour	Intensity of roast beef flavour during mastication	Low–high
Metallic	Flavour associated with iron or blood	Not–very
Stale/rancid/aged	Flavour of stale, aged and gone off beef	Not–very
Swallow the beef and wait for 12 s before scoring the following aftereffects		
<b>Aftereffect</b>		
Roast beef flavour length	Intensity of beef flavour left 12 s after swallowing the sample	Not–very
Metallic	Flavour associated with iron or blood after swallowing	Not–very
Fattiness/greasiness	Fatty mouth coating after swallowing the sample	Not–very
Dryness	Dry mouth feel after swallowing the sample	Not–very

<sup>1</sup>First terms in the anchors indicate the left end of the scale, and the second term anchors indicate the right end.

(25, 19 and 22 mo), first-season feeding (PO and PC) as described earlier and their interactions. Multiple comparisons were adjusted by the Tukey–Kramer test with a significance level of  $P < 0.05$ , whereas differences of  $P > 0.05$  to  $P < 0.10$  were considered as trends. Residual Pearson correlation coefficients (after data were adjusted for the fixed effects in the model) between variables were calculated using the CORR procedure of SAS (2002).

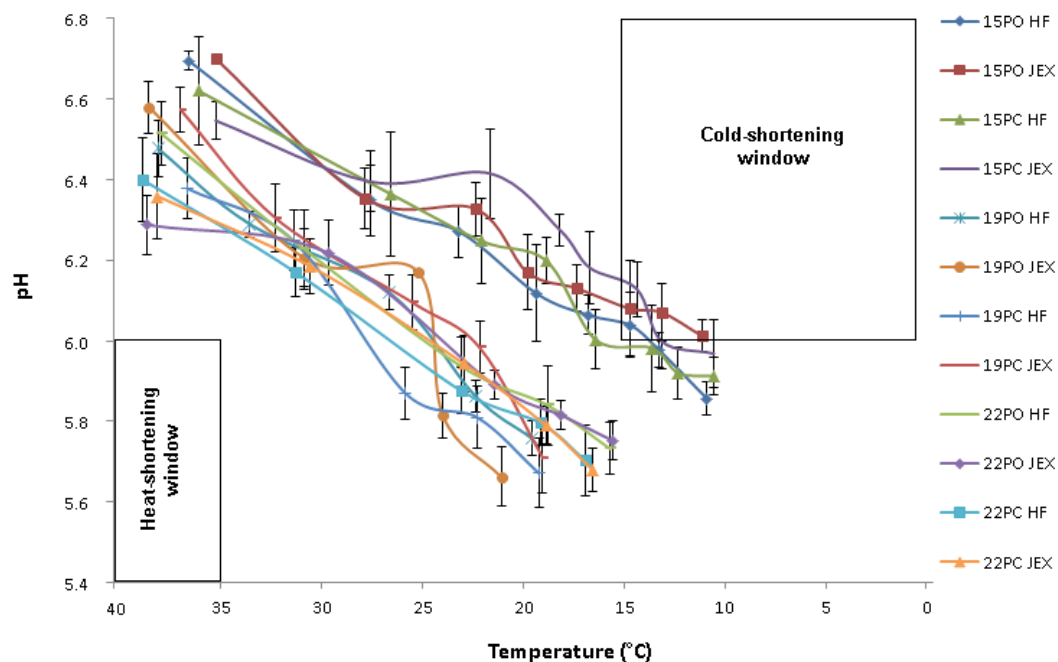
## Results and discussion

### Post-mortem pH/temperature decline and pH<sub>u</sub>

The pH/temperature window concept implemented in the Meat Standards Australia (MSA) grading scheme was used to monitor or identify carcasses at risk of cold shortening with pH > 6 at temperature < 15°C or heat toughening with pH < 6 at temperature > 35°C. Cold shortening occurs

while adenosine triphosphate (ATP) is available for muscle contraction, whereas heat toughening occurs when the activity of proteolytic enzymes is exhausted within the muscle, thereby reducing the ageing potential (Thompson, 2002). In this study, the only group of carcasses falling inside the cold-shortening window was that comprising the 15-mo-old dairy bulls (two JEX and one HF) and no groups were inside the heat-shortening window (Figure 2). This was probably due to the faster chilling of the lighter carcasses of 15-mo-old bulls, particularly the JEX bulls.

The pH at 15°C and 35°C were the highest for 15-mo-old bulls ( $P < 0.001$ ; Table 2). Likewise, at each hour post mortem, younger animals had higher pH values than older ones, probably due to their faster chilling rate resulting from insufficient subcutaneous fat cover. Early post-mortem pH contributes to meat tenderness as it affects the activity of endogenous enzymes, with a pH<sub>u</sub> of 6.0–6.1, reflecting the optimum glycolytic rate to give the most tender meat (Chambaz *et al.*, 2003), while individual carcass pH<sub>u</sub> values in this study



**Figure 2.** Post-mortem pH and temperature decline of young dairy bulls up to 8 h after slaughter (average for the animals in each production group). 15, 19, 22 = slaughter age (in mo). PO = grass only during the first grazing season; PC = grass plus 2 kg concentrate during the first grazing season. HF = Holstein–Friesian; JEX = Jersey × HF. Error bars = standard errors of mean values. Cold- and heat-shortening windows are according to the review of Thompson (2002).

**Table 2.** pH at 15°C and 35°C, pHu and colour after 2 h and 24 h of blooming of the *longissimus thoracis* muscles of young dairy bulls

Physical attributes	Age, mo (A)						Breed (B)				First season (F)				P-value		
	15 (n = 29)		19 (n = 19)		22 (n = 19)		HF (n = 39)		JEX (n = 28)		Concentrates (n = 34)		Pasture (n = 33)		A	B	F
	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.			
pH (at 15°C) <sup>1</sup>	6.05 <sup>a</sup>	0.04	5.48 <sup>c</sup>	0.04	5.69 <sup>b</sup>	0.04	5.73	0.03	5.74	0.04	5.75	0.03	5.73	0.03	<.001		
pH (at 35°C) <sup>2</sup>	6.65 <sup>a</sup>	0.05	6.40 <sup>b</sup>	0.06	6.30 <sup>b</sup>	0.06	6.44	0.04	6.46	0.05	6.46	0.04	6.44	0.04	<.001		
pHu <sup>3</sup>	5.73 <sup>a</sup>	0.02	5.55 <sup>b</sup>	0.03	5.57 <sup>b</sup>	0.02	5.62	0.02	5.61	0.02	5.60	0.02	5.62	0.02	<.001		
L* at 2 h	45.1 <sup>a</sup>	0.60	42.9 <sup>b</sup>	0.62	42.1 <sup>b</sup>	0.61	44.4 <sup>a</sup>	0.46	42.4 <sup>b</sup>	0.53	43.0	0.50	43.7	0.49	0.003	0.006	
a* at 2 h	16.4	0.37	15.9	0.38	16.2	0.37	15.7 <sup>b</sup>	0.28	16.7 <sup>a</sup>	0.32	16.4	0.31	16.0	0.30		0.030	
b* at 2 h	13.2	0.31	12.6	0.32	12.6	0.31	13.1	0.24	12.4	0.27	12.7	0.26	12.9	0.25		0.058	
Hue angle at 2 h	38.9	0.64	38.4	0.66	37.7	0.64	39.9 <sup>a</sup>	0.49	36.7 <sup>c</sup>	0.56	37.8	0.54	38.9	0.52		<.001	
Saturation at 2 h	21.1	0.42	20.3	0.44	20.5	0.43	20.5	0.33	20.8	0.37	20.7	0.35	20.6	0.34			
L* at 24 h	45.2 <sup>a</sup>	0.47	43.4 <sup>b</sup>	0.49	42.0 <sup>b</sup>	0.47	44.3 <sup>a</sup>	0.36	42.8 <sup>b</sup>	0.41	43.0	0.39	44.1	0.38	<.001	0.011	0.067
a* at 24 h	17.6 <sup>a</sup>	0.47	15.7 <sup>b</sup>	0.49	17.5 <sup>a</sup>	0.47	16.7	0.36	17.2	0.41	17.2	0.39	16.7	0.38	0.010		
b* at 24 h	14.0 <sup>a</sup>	0.31	12.1 <sup>b</sup>	0.32	13.4 <sup>a</sup>	0.32	13.5	0.24	12.8	0.28	13.0	0.26	13.3	0.25	0.001	0.073	
Hue angle at 24 h	38.6	0.59	37.7	0.62	37.4	0.60	39.0 <sup>a</sup>	0.46	36.8 <sup>b</sup>	0.52	37.2	0.50	38.5	0.48		0.003	0.067
Saturation at 24 h	22.5 <sup>a</sup>	0.52	19.8 <sup>b</sup>	0.54	22.1 <sup>a</sup>	0.52	21.5	0.40	21.4	0.46	21.6	0.44	21.3	0.42	0.002		

<sup>a-c</sup>Mean values within a row within a main effect with different superscripts significantly differ ( $P < 0.05$ ).

<sup>1</sup>pH (at 15°C) = pH at the temperature of 15°C.

<sup>2</sup>pH (at 35°C) = pH at the temperature of 35°C.

<sup>3</sup>pHu = ultimate pH. LSM = least square mean values.



ranged from 5.87 to 6.42, suggesting that tenderness would be variable.

The pH<sub>u</sub> was higher for 15-mo-old bulls ( $P < 0.001$ ), while 19- and 22-mo-old bulls were similar (Table 2). The pH<sub>u</sub> did not differ between breed types or first-season feeding ( $P > 0.05$ ). Mean pH<sub>u</sub> values for all groups ranged from 5.53 to 5.74, which lie within the range considered normal for beef (Tarrant, 1989). No dark, firm and dry (pH<sub>u</sub> > 6.0) meat was observed.

### Meat colour

CIE L\* after both 2 h ( $P < 0.01$ ) and 24 h ( $P < 0.001$ ) blooming decreased with slaughter age (Table 2), in agreement with the results of others (Page *et al.*, 2001; Serra *et al.*, 2004), indicating that meat from older animals was darker than meat from younger animals. Gil *et al.* (2001) found that muscle pigment content increased with age, which is consistent with meat from older animals being darker. JEX beef was darker than HF after both 2 h ( $P < 0.01$ ) and 24 h ( $P < 0.05$ ) blooming. The paler appearance of HF beef was probably due to increased light scattering, related to their higher cooking loss. Water loss during cooking occurs mainly from the juice expelled by myofibrillar lattice shrinkage caused by protein denaturation. Higher extent of muscle fibre shrinkage also creates larger gaps between fibres, which could allow for increased light scattering (Hughes *et al.*, 2014). JEX bull beef was redder after 2 h of blooming ( $P < 0.05$ ). Likewise, the greater hue angle in HF after both 2 h ( $P < 0.001$ ) and 24 h ( $P < 0.01$ ) of blooming compared to JEX beef indicates a less pure red colour in HF. This is possibly due to the higher WHC of JEX beef, resulting in more myoglobin remaining after chilling for 24 h (Waritthitham *et al.*, 2010). The metmyoglobin reducing activity, or the amount of its essential cofactor reduced nicotinamide adenine dinucleotide (NADH), can also cause differences in redness development in meat between breeds (Bekhit and Faustman, 2005).

### WBSF analysis

Generally, it is recommended to age steak for at least 14 d to ensure tender beef (Farouk *et al.*, 2009). Monsón *et al.* (2005) suggested that longer ageing periods (21 d) would be needed for Holstein beef to attain optimum acceptability. Hence, WB variables and sensory traits of steaks aged for 21 d were investigated.

WB slope and area are two other instrumental variables related to sensory texture attributes. WB slope or modulus was calculated to express “shear firmness”, with higher values corresponding to lower elasticity (Brady and Hunecke, 1985). Total energy, corresponding to the total area under the WBSF curve, was used to describe the total energy consumed to chew the meat until it could be swallowed. It is hypothesised that the initial yield force occurring before maximum peak force is probably associated with the myofibrillar component and

the final yield after maximum shear force corresponds to the connective tissue component (Moller, 1981). Consequently, the first peak of the WB curve is related to the contribution of the myofibrillar structure to toughness.

WBSF, WB slope, WB area and WB first peak force were unaffected by breed type, age and first-season feeding ( $P > 0.05$ ; Table 3). The 15-mo-old dairy bulls did not produce more tender meat than the 19- and/or 22-mo-old bulls, in agreement with Sinclair *et al.* (1998). However, this is in contrast to Dransfield *et al.* (2003), who found that beef from 15-mo-old bulls was more tender than beef from 19- or 24-mo-old bulls, which had similar tenderness levels. It is likely that, in the current study, cold shortening occurred in some of the 15-mo-old bulls and increased the mean WBSF of that group. The relatively narrow range in WBSF values may be due to the extended post-mortem ageing of 21 d, which may also have contributed to removing any age effect. It was shown by Jurie *et al.* (2005) that differences in steak WBSF values between breeds disappeared after 14 d of ageing.

WBSF varied from 17.37 to 46.08 N for LT steaks aged for 21 d. Shackelford *et al.* (1991) categorised muscle groups into “very tender” (WBSF < 31.36 N), “tender” (31.36 < WBSF < 38.22 N), “intermediate” (38.22 < WBSF < 45.08 N) and “tough” (WBSF > 45.08 N). Based on this classification, only four animals could be considered to be of intermediate toughness, and only one animal could be considered tough. All other samples were in the very tender and tender categories. Likewise, Tatum *et al.* (1999) set the WBSF limit of 44.5 N as unacceptably tender beef. In addition, a 98% acceptability rating by American consumers would correspond to a WBSF value equal to, or less than, 40.18 N (Huffman *et al.*, 1996). The mean WBSF value for the 12 groups investigated in this study ranged from 25.76 N (22-mo-old JEX bulls from PO) to 33.09 N (15-mo-old JEX bulls from PO). Therefore, LT steaks from HF and JEX bulls were considered tender after 21 d of ageing.

### Cooking loss

Cooking loss was affected only by breed type, with JEX bull beef having lower cooking loss than HF bull beef ( $P < 0.01$ ; Table 3). The mean values of individual groups varied between 26.6% and 30.9%. Pordomingo *et al.* (2012) found that muscles with higher IMF content have lower cooking loss. Even though there was no difference between the breed types in terms of IMF content in this study, in the sensory evaluation, beef from JEX bulls was found to be more fatty/greasy than that from HF ( $P < 0.05$ ; Table 4).

### Chemical composition

Moisture and ash contents were higher in beef from 15- than 22-mo-old bulls ( $P < 0.05$ ), while IMF and protein content were unaffected by slaughter age ( $P > 0.05$ ; Table 3). Pflanzner

**Table 3.** WB variables, cooking loss, chemical composition and collagen characteristics of *longissimus thoracis* muscles of young dairy bulls

Characteristics	Age, mo (A)						Breed (B)				First season (F)				P-value		
	15 (n = 29)		19 (n = 19)		22 (n = 19)		HF (n = 39)		JEX (n = 28)		Concentrates (n = 34)		Pasture (n = 33)		A	B	F
	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	SEM			
WBSF <sup>1</sup> (N)	29.6	1.15	29.8	1.39	27.7	1.38	28.4	1.01	29.6	1.13	28.9	1.09	29.1	1.05			
WB slope (Mpa)	0.56	0.03	0.56	0.03	0.50	0.03	0.54	0.02	0.54	0.03	0.55	0.03	0.53	0.02			
WB area (J)	0.28	0.01	0.28	0.01	0.27	0.01	0.27	0.01	0.29	0.01	0.28	0.01	0.28	0.01			
WB first peak force (N)	23.6	0.84	26.0	1.02	24.6	1.01	24.5	0.74	24.9	0.83	24.8	0.80	24.7	0.77			
Cooking loss (%)	29.3	0.49	28.7	0.59	28.8	0.59	29.9 <sup>a</sup>	0.43	27.9 <sup>b</sup>	0.48	29.3	0.46	28.5	0.45		0.003	
Moisture (%)	73.3 <sup>a</sup>	0.20	72.6 <sup>ab</sup>	0.24	72.4 <sup>b</sup>	0.24	72.9	0.17	72.7	0.19	72.7	0.19	72.9	0.18	0.013		
IMF <sup>2</sup> (%)	2.76	0.26	3.34	0.31	3.26	0.31	3.08	0.23	3.15	0.25	3.28	0.24	2.95	0.24			
Protein (%)	22.6	0.16	22.3	0.20	22.7	0.19	22.5	0.14	22.6	0.16	22.5	0.15	22.6	0.15			
Ash (%)	1.07 <sup>a</sup>	0.01	1.05 <sup>ab</sup>	0.01	1.03 <sup>b</sup>	0.01	1.05	0.01	1.05	0.01	1.05	0.01	1.05	0.01	0.049		
Soluble collagen (mg/g)	0.91	0.03	0.89	0.04	0.85	0.04	0.88	0.03	0.89	0.03	0.88	0.03	0.89	0.03			
Insoluble collagen (mg/g)	5.84	0.31	5.52	0.39	5.63	0.40	5.53	0.28	5.80	0.32	5.67	0.30	5.66	0.31			
Total collagen (mg/g)	6.75	0.34	6.41	0.42	6.48	0.43	6.41	0.30	6.68	0.35	6.55	0.32	6.55	0.33			
Collagen solubility (%)	13.9	0.52	14.5	0.65	13.4	0.67	14.1	0.47	13.7	0.54	13.8	0.50	14.0	0.51			

<sup>a,b</sup>Mean values within a row within a main effect with different superscripts significantly differ ( $P < 0.05$ ).

<sup>1</sup>WBSF = Warner-Bratzler shear force.

<sup>2</sup>IMF = Intramuscular fat. LSM = least square mean values.

**Table 4.** Sensory evaluation of *longissimus thoracis* muscles of young dairy bulls

Sensory attributes	Age, mo (A)						Breed (B)				First season (F)				P-value		
	15 (n = 29)		19 (n = 19)		22 (n = 19)		HF (n = 39)		JEX (n = 28)		Concentrates (n = 34)		Pasture (n = 33)		A	B	F
	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.	LSM	s.e.			
Roast beef aroma	56.5	1.70	63.7	2.70	60.0	2.24	58.5	1.61	61.7	2.04	61.9	2.01	58.3	1.64	0.080		
Initial tenderness	72.7	2.43	69.4	3.84	69.5	3.19	67.4	2.29	73.6	2.91	71.4	2.86	69.6	2.34		0.100	
Juiciness	51.0	2.90	48.8	4.59	41.0	3.81	44.3	2.74	49.5	3.47	49.7	3.42	44.1	2.80			
Cohesiveness	53.4	2.50	56.2	3.95	52.7	3.28	55.1	2.36	53.1	2.99	53.6	2.95	54.6	2.41			
Ease of disintegration	74.9	2.27	66.9	3.60	70.7	2.99	69.5	2.15	72.1	2.72	69.9	2.68	71.8	2.2			
Chewiness	29.5	2.67	34.4	4.22	31.1	3.50	32.6	2.52	30.7	3.19	31.4	3.15	32.0	2.57			
Fattiness/greasiness	15.1	0.97	14.5	1.53	14.7	1.27	13.0 <sup>b</sup>	0.91	16.5 <sup>a</sup>	1.16	15.2	1.14	14.3	0.93		0.022	
Stringiness	11.5	1.51	15.0	2.39	15.0	1.98	15.5	1.43	12.2	1.81	15.8	1.78	11.9	1.46			
Astringency	16.0	1.60	17.3	2.54	20.7	2.10	17.7	1.51	18.2	1.92	18.0	1.89	18.0	1.55			
Roast beef flavour	54.0	1.91	57.0	3.02	56.6	2.51	54.2	1.80	57.5	2.29	53.5	2.25	58.2	1.84			
Metallic taste	12.8	1.88	17.5	2.98	16.3	2.47	13.0	1.78	18.1	2.25	16.2	2.22	14.9	1.81		0.086	
Stale/rancid/aged	3.45	0.63	4.94	1.00	4.13	0.83	3.55	0.60	4.80	0.76	4.98	0.75	3.37	0.61			
Res <sup>1</sup> -RBFL <sup>2</sup>	47.9 <sup>b</sup>	1.71	50.7 <sup>ab</sup>	2.70	55.1 <sup>a</sup>	2.24	49.9	1.61	52.6	2.04	49.4	2.01	53.0	1.65	0.048		
Res <sup>1</sup> -metallic	12.3	1.85	17.3	2.92	17.9	2.42	14.3	1.74	17.4	2.21	17.6	2.18	14.0	1.78			
Res <sup>1</sup> -fattiness/greasiness	16.7	1.13	17.5	1.80	16.4	1.49	16.1	1.07	17.6	1.36	17.8	1.34	15.9	1.09			
Res <sup>1</sup> -dryness	16.7	1.62	17.6	2.56	17.7	2.13	18.3	1.53	16.4	1.94	16.4	1.91	18.2	1.56			

<sup>a,b</sup>Mean values within a row within a main effect with different superscripts significantly differ ( $P < 0.05$ ).

<sup>1</sup>Res = residual (aftereffects).

<sup>2</sup>Res-RBFL = Res-roast beef flavour length. LSM = least square mean value.



and de Felício (2011) also found that beef from lower-maturity animals had more moisture than those from more mature animals. Chemical composition parameters were similar for HF and JEX bull beef and for the two first-season treatments ( $P > 0.05$ ; Table 3). Mean IMF content in all groups ranged from 2.02% to 4.04%, which was within the range from 0.45% to 6.65% indicated in Waritthitham *et al.* (2010) for IMF content among beef breeds. The average IMF content of LT muscle observed here was low ( $< 5\%$ ) and this finding is in agreement with other reports (O'Neill *et al.*, 2004; Riley *et al.*, 2005; Serra *et al.*, 2008). An IMF level of approximately 3.25% was defined as a “slight degree of marbling” grade and was reported to be preferred by US consumers on visual quality (Killinger *et al.*, 2000). Most (47%) Swiss consumers preferred beef with 3%–4% IMF; however, 27% selected beef with no visible marbling (Chambaz *et al.*, 2003). According to these authors, IMF  $< 3\%$  was considered to result in tougher, drier and less flavourful meat by most consumers. Approximately one-half ( $n = 32$ ) of the samples had IMF values  $> 3\%$  and, therefore, would be in the acceptable range for most consumers.

#### **Collagen content and solubility**

The total amount and chemical composition of collagen is believed to primarily determine the “background” toughness of beef after prolonged ageing. It is generally accepted that higher levels of intramuscular connective tissue, particularly the more mature cross-links, are associated with reduced beef tenderness (Jeremiah *et al.*, 2003). Collagen content and solubility were unaffected by breed type, slaughter age and first-season feeding ( $P > 0.05$ ; Table 3). Collagen solubility during heat treatment depends on the number and extent of multivalent mature cross-links present between tropocollagens (Bailey, 1985). The accumulation of intermolecular cross-links accelerates with age, decreasing collagen thermal solubility (Weston *et al.*, 2002). The relatively narrow range of animal age in this study may have been insufficient to result in differences in collagen characteristics. Dransfield (1977) reported that the strong relationship between collagen content and cooked meat tenderness is mainly from samples with large variation in collagen content, e.g. intermuscular comparisons. Shorthouse and Harris (1990) pointed out that collagen-rich muscles were more likely to show age-associated toughness of beef. It is important to note that in the current study, only the LT, a relatively tender muscle, was used, which further reduced the potential for variation in collagen content and solubility. Similarly, a lack of any effect of age on the collagen content of the *longissimus dorsi* (LD) muscle from bulls and steers slaughtered at four ages was reported by Dikeman *et al.* (1986). Schönfeldt and Strydom (2011) also showed that age had no effect on the collagen content of South African cattle. The lack of a diet effect on collagen is in accordance with the results of Dikeman *et al.* (1986).

Mean total and insoluble collagen contents observed in all groups ranged from 6.06 to 7.09 mg/g wet tissue and from 5.16 to 6.21 mg/g, respectively, while collagen solubility ranged from 12.62% to 15.27%. A previous study showed that LT muscle from Jersey and Holstein bulls of 13–16 mo old had 4.07 and 3.86 mg/g wet tissue of total collagen, 2.96 and 3.02 mg/g of insoluble collagen and collagen solubility of 27.3% and 21.7%, respectively (Christensen *et al.*, 2011). The higher contents of both total and insoluble collagen and the lower collagen solubility determined in the current study probably resulted from the different collagen determination method applied. Christensen *et al.* (2011) concluded that among most cattle breeds in Europe, the dairy breeds Jersey, Holstein and Danish Red have the highest total and insoluble collagen content, while the meat breeds Piedmontese, Limousin and Asturiana de los Valles have the lowest values. However, these authors also determined that the percentage of heat-soluble collagen was highest in Jersey beef but lowest in Danish Red and Holstein beef when compared to all breeds investigated in their study. This may explain why HF beef was judged as relatively tougher than JEX beef by the sensory panel in the current study.

#### **Evaluation by the trained sensory panel**

With increasing slaughter age, residual roast beef flavour length increased ( $P < 0.05$ ; Table 4). This was expected as Lawrie (1991a) and Dransfield *et al.* (2003) reported that flavour intensity increased with animal age. Intensity of fattiness/greasiness was higher in JEX beef ( $P < 0.05$ ), in accordance with the finding that Jersey cattle tend to produce a highly marbled product (Albertí *et al.*, 2008), even though there was no difference in IMF content in the current study. Riley *et al.* (1986) found no differences in WBSF and overall palatability between Jersey-type and Holstein-type bull beef, while in this study JEX beef tended to have higher initial tenderness than HF beef ( $P = 0.10$ ), which may be related to the higher WHC represented by less cooking loss. The difference in tenderness could also be due to variation in calpain and calpastatin activity, contributing to variation in the rate and extent of muscle proteolysis during the post-mortem ageing period (Lawrie, 1991b).

Rancidity is an off-flavour resulting from enzymatic degradation processes and lipid peroxidation of unsaturated fatty acids, which can occur in meat during ageing (Wood *et al.*, 2003). The rancid flavour score in our study was surprisingly low ( $< 5$ ) considering the long ageing period adopted (21 d) and was probably due to the storage of beef samples under vacuum, thus reducing the rate of lipid oxidation (Resconi *et al.*, 2010). The mean overall scores for beef flavour and juiciness were 56 and 47, respectively, in this study, in agreement with the sensory scores of young Friesian bulls reported by Partida *et al.* (2007). Initial tenderness score was higher (around 70)

in this study, indicating that tender beef can be produced from dairy bulls after ageing for 21 d.

#### Residual correlations between variables

The pH (35°C) was negatively correlated with WB first peak force ( $P < 0.01$ ; Table 5), which indicated that heat shortening increases myofibrillar toughness of beef. The  $a^*$  value after 2 h of blooming was negatively correlated with WB slope and cooking loss but positively correlated with soluble collagen content ( $P < 0.05$ ). WB variables (including WBSF, WB slope and WB area) were positively correlated with cooking loss ( $P < 0.05$ ), which agreed with the results of Monteiro *et al.* (2013). WB slope was negatively correlated with IMF content but positively correlated with moisture content ( $P < 0.05$ ), which agreed with the finding that a decreased WBSF was associated with increased marbling (Li *et al.*, 2006). WBSF and WB slope were negatively correlated with soluble collagen content ( $P < 0.05$ ). Samples with higher cooking loss had higher moisture content ( $P < 0.05$ ), which is in accordance with the results of Chambaz *et al.* (2003). Higher

cooking loss related to the decreased marbling level ( $P < 0.01$ ), which is partially attributed to the melting of fat by heat, as it protects against moisture loss of steaks during cooking. WB variables were positively correlated with cohesiveness ( $P < 0.001$ ), chewiness and stringiness ( $P < 0.05$ ) and were negatively correlated with initial tenderness and ease of disintegration ( $P < 0.05$ ; Table 6). The correlations between WBSF and sensory tenderness are in agreement with other reports (Chambaz *et al.*, 2003; Schönfeldt and Strydom, 2011; Monteiro *et al.*, 2013). IMF content was positively correlated with initial tenderness ( $P < 0.01$ ) and negatively correlated with cohesiveness ( $P < 0.05$ ), in agreement with several reports that meat tenderness can be improved by IMF content (Savell and Cross, 1988; Sami *et al.*, 2004; Corbin *et al.*, 2015) as IMF dilutes the fibrous protein in muscle tissue, resulting in a decrease in muscle resistance to shearing (Wood *et al.*, 1999).

Soluble collagen content was positively correlated with initial tenderness ( $P < 0.01$ ) and ease of disintegration ( $P < 0.05$ ) and was negatively correlated with stringiness ( $P < 0.01$ ; Table 6).

**Table 5.** Residual Pearson correlation coefficients between physico-chemical traits of *longissimus thoracis* muscles of young dairy bulls

Traits	WBSF	WB slope	WB area	WB first peak force	Cooking loss	IMF	Moisture	Soluble collagen
pH (at 15°C)	-0.16	-0.05	-0.10	-0.10	0.09	-0.14	0.09	0.01
pH (at 35°C)	-0.15	-0.20	-0.07	-0.33**	-0.17	0.22	-0.21	-0.03
L* at 2 h	0.14	0.01	0.20	-0.13	0.15	0.06	-0.06	-0.18
a* at 2 h	-0.21	-0.29*	-0.24	-0.08	-0.28*	0.09	-0.06	0.33*
WBSF <sup>1</sup>		0.83***	0.92***	0.68***	0.32**	-0.14	0.11	-0.27*
WB slope			0.67***	0.64***	0.27*	-0.29*	0.25*	-0.39*
WB area				0.57***	0.36**	-0.11	0.11	-0.24
Cooking loss						-0.31**	0.26*	-0.17
IMF <sup>2</sup>							-0.91***	0.19

<sup>1</sup>WBSF = Warner-Bratzler shear force.

<sup>2</sup>IMF = Intramuscular fat.

**Table 6.** Residual Pearson correlation coefficients between physico-chemical and sensory traits of *longissimus thoracis* muscles of young dairy bulls

Traits	Initial tenderness	Ease of disintegration	Cohesiveness	Chewiness	Stringiness	Juiciness
WBSF <sup>1</sup>	-0.44***	-0.36**	0.52***	0.47***	0.29*	-0.11
WB slope	-0.56***	-0.52***	0.59***	0.56***	0.48***	-0.10
WB area	-0.33*	-0.28*	0.47***	0.42**	0.17	-0.15
WB first peak force	-0.40**	-0.33*	0.50***	0.35*	0.14	0.07
IMF <sup>2</sup>	0.39**	0.24	-0.28*	-0.22	-0.24	0.03
Soluble collagen	0.39**	0.30*	-0.25	-0.19	-0.39**	0.26
Initial tenderness	1.00	0.69***	-0.51***	-0.70***	-0.68***	0.37**
Roast beef flavour	0.32*	0.23	-0.12	-0.19	-0.24	0.42**
Chewiness	-0.70***	-0.78***	0.56***	1.00	0.62***	-0.32*

<sup>1</sup>WBSF = Warner-Bratzler shear force.

<sup>2</sup>IMF = Intramuscular fat.

Stringiness means fibrous nature of meat with long, thin string-like pieces that are hard to chew; thus, it relates to chewiness. However, in this study, no correlations between total collagen content and WB variables or sensory tenderness parameters were found ( $P > 0.05$ ). This corresponds with other findings that while total collagen content is not directly associated with objective and sensory texture characteristics, the percentage of soluble collagen was the main determinant of tenderness and ease of fibre fragmentation by the sensory panel (Bailey, 1985; Schönfeldt and Strydom, 2011).

Juiciness was positively correlated with initial tenderness ( $P < 0.01$ ) and negatively correlated with chewiness ( $P < 0.05$ ; Table 6), which is in agreement with other studies (e.g. Serra *et al.*, 2008; Monteiro *et al.*, 2013). This was probably a result of juices being released more quickly through chewing more tender meat, thereby contributing to a juicier meat sensation (Savell and Cross, 1988). Roast beef flavour was positively correlated with juiciness ( $P < 0.01$ ), in agreement with the result of Monteiro *et al.* (2013). A positive correlation was also observed between roast beef flavour and initial tenderness score ( $P < 0.05$ ), which was in accordance with the finding by Gill *et al.* (2010). This effect could be due to the “halo effect”, i.e. when only a few attributes of a product are evaluated, the ratings will tend to influence each other (Meilgaard *et al.*, 1999). Likewise, initial tenderness had a high positive relationship with ease of disintegration and negative relationships with cohesiveness, chewiness and stringiness ( $P < 0.001$ ). It seems that when a panellist rated a piece of tender meat, he/she was also prone to giving higher scores to other traits, particularly the unrelated ones, such as flavour (Gill *et al.*, 2010).

## Conclusions

The eating quality of beef from the young dairy bulls investigated in this study was generally good after 21 d of ageing. Only three of the samples had a WBSF score  $> 40$  N, indicating that most beef samples were acceptably tender. Some eating quality attributes were affected by breed type and age at slaughter, but first-season feeding had no effect. With age at slaughter increasing from 15 to 22 mo, beef became darker, moisture decreased and the meat was judged to hold the beef flavour longer during sensory evaluation. JEX beef had lower cooking loss, was more greasy and was relatively tender compared to HF beef. Therefore, these data suggest that crossbreeding the Jersey breed with HF might improve the beef quality of young dairy bulls, though a larger-scale study would be needed to definitively establish the relative merits of the two breeds. Variations in cooking loss, IMF and soluble collagen content could explain the differences determined in the cooked meat texture. Higher WB variables (WB slope, WB

area and WB first peak force) were associated with higher sensory cohesiveness and chewiness scores, as well as lower scores for initial tenderness and ease of disintegration. The effects on colour could be important in terms of selecting carcasses for different markets. Marbling level also had an influence on WHC and other characteristics such as cooking loss and sensory texture parameters of young dairy bull beef and, based on these results, crossing HF cows with Jersey bulls or slaughtering earlier (15 vs. 19 or 22 mo) would not be expected to reduce the IMF content. It can be concluded that good-quality beef can be produced from young dairy bulls of different breed types.

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## Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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